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Cloning and chromosomal localization of the gene coding for human protein kinase CK1

Claudio Tapia[a], Terence Featherstone[b], Claudio Gómez[a], Patricia Taillon-Miller[a], Catherine C. Allende[c], Jorge E. Allende[a,*]

[a]*Departamento de Bioquímica, Facultad de Medicina, Universidad de Chile, Casilla 70086, Santiago 7, Chile*
[b]*Department of Genetics, Washington University School of Medicine, St. Louis, MO 63110, USA*
[c]*Departamento de Biología, Facultad de Ciencias, Universidad de Chile, Chile*

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Abstract

A cDNA clone coding for human protein kinase CK1 (casein kinase 1) has been isolated and sequenced demonstrating that it corresponds to a homolog of the CK1α form found in bovine brain. The derived amino acid sequence of the human CK1α is identical to the bovine counterpart except that it contains 12 extra amino acids at the carboxyl end. Using this cDNA sequence and PCR amplification, YAC genomic clones that contain this human CK1α sequence have been isolated. These YACs have been used for fluorescent in situ hybridization in order to localize the human CK1α gene to chromosome 13q13.

Key words: Casein kinase 1; Chromosome 13; Protein kinase; Gene mapping; Fluorescent in situ hybridization

1. Introduction

Protein kinase CK1 (previously known as casein kinase 1) is a ubiquitous ser/thr protein kinase that has been found in the nucleus, cytoplasm and membrane fractions of eukaryotic cells (see reviews [1–2]). This abundant enzyme is a monomeric (34–55 kDa) protein that does not seem to be regulated by the well-known second messengers involved in signal transduction.

Several findings contribute to the interest in studying this enzyme. It has been observed, for instance, that CK1 phosphorylates a number of important proteins such as RNA polymerases I and II, the SV-40 large T antigen, the p53 tumor suppressor protein and the mRNA cap-binding protein [2]. CK1 has also been reported to participate in the hierarchal phosphorylation of glycogen synthase with the cAMP-dependent protein kinase [3].

Immunofluorescent studies using mouse fibroblasts and CHO cells localized CK1 in vesicular structures during interphase. During mitosis, however, this enzyme is concentrated in the mitotic spindle, in the centrosome as well as in the kinetochore microtubules [4]. Also relevant to a possible involvement of CK1 in cell division is the finding that a yeast mutant (HRR25) which was found to cause defects in DNA strand-break repair has turned out to be highly homologous to CK1 [5]. HRR25 mutants are also defective in meiosis and show delays in G_2–M phase transition in the cell cycle.

Several genes coding for CK1 have been described [6–8]. In yeast the genes YCK1 and YCK2 were isolated independently. YCK1 was isolated as a suppressor of the requirement of SNF4 function, while increased dosage of YCK2 allows cells to survive extreme salinity. These two genes were found to be very similar to each other and 64% identical to a fraction of rabbit CK1. Loss of function of either of these genes shows no detectable phenotype but when both genes are lost the cells are not viable [6]. The deduced proteins from these genes have estimated M_r of 61,000–62,000, which is significantly larger than the enzyme found in animal cells.

In bovine brain, cDNAs coding for 4 different but homologous subspecies of CK1 have been reported [7]. The α and β subspecies were 37 and 38 kDa proteins, respectively. In rat testis, a cDNA clone was isolated which coded for a 49 kDa casein kinase subspecies called δ, which is 76% identical to bovine α and 75% identical to HRR25 [8].

Recently, our laboratory has cloned cDNA encoding a *Xenopus laevis* casein kinase 1 which is very similar to the bovine α subspecies (Tapia, C. et al., manuscript in preparation). Partial amino acid sequences obtained from purified peptides of human CK1 were reported [4]. These *X. laevis* and human amino acid sequences are identical to the bovine CK1α subspecies.

This report presents the cloning and sequence of a cDNA containing the complete coding region of the human CK1 gene which was found to be highly homologous to the bovine CK1α amino acid sequence. Also yeast artificial chromosomes (YACs) containing human inserts, that include the same or homologous CK1α sequence have been identified and used to localize the gene

*Corresponding author. Fax: (56) (2) 737 6320.

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through fluorescent in situ hybridization to chromosome region 13q13.

2. Materials and methods

2.1. Cloning of the human CK1α cDNA

Primers were synthesized on the basis of the published bovine sequences that are common to all CK1 species [7]. These primers had the sequences 5′-CCC AGA TAA CTT CCT AAG GGG-3′ and 5′-AGC CTT TAG TCC TTC CAT GGA-3′ and were used for PCR amplification of human fetal brain λZAP (Stratagene) cDNA library. A fragment of 264 base pairs was obtained and sequenced, demonstrating that it corresponded to a human CK1 gene due to its homology to the bovine sequence. This PCR fragment was labeled by random oligonucleotide primers [^{32}P]dATP yielding a specific activity of 10^9 cpm/μg and used as a probe to screen 5×10^5 clones of the same human cDNA library. Seventeen clones were obtained after tertiary screening. Analysis of these clones demonstrated that only 8 had the complete coding region, the other ones being truncated.

The size of the insert in one of the complete clones was approximately of 1,600 bp. This clone was rescued from the λZAP phage to yield a Bluescript plasmid. This plasmid was purified and sequenced by the dideoxy method [9].

2.2. Screening of the YAC library

Primers were designed using an oligonucleotide selection program [10] on the published bovine CK1α cDNA sequence [7]. The chosen primers were 5′-CTT AAA CTA TTG TCG TGG G-3′ and 3′-TCA TAT TGG TGA TTC AGG G-5′ corresponding to 1,170–1,188 and 1,271–1,253 of the published sequence of bovine CK1 and giving a product length of 102 bp.

Screening of the YAC libraries was done entirely by PCR using standard methods [11,12]. Screening was done in the Center for Genetics in Medicine at Washington University by a robot-assisted hierarchical identification scheme [13]. Two YAC libraries containing approximately 5 genome equivalents were screened with the chosen primer pair. These libraries were both made from human lymphoblastoid cell lines (48, XXXX) [14] and (49, XXXXX) (Schlessinger, D., personal communication).

2.3. Alu-PCR and DNA labeling

Alu-PCR was done on yeast clones as described by Lengauer, et al. [15]. Briefly, agarose plugs containing yeast clones with human insert YACs were equilibrated extensively in PCR-buffer (10 mM Tris-HCl, pH 8.4, 50 mM KCl, 1.5 mM $MgCl_2$). Using 0.25 μM each of the primers CL1 (5′-TCC CAA AGT GCT GGG ATT ACA G-3′) and CL2 (5′-CTG CAC TCC AGC CTG GG-3′) PCR was carried out in PCR-buffer containing 100 ng DNA from a melted plug, 250 μM dNTPs and 5 units Taq polymerase (Perkin Elmer/Cetus). After initial denaturation at 94°C for 3 min, 30 cycles of PCR were carried out with denaturation at 94°C for 1 min, a 2 min 30 s ramp to a 30 s annealing at 37°C, a 45 s ramp to a 6 min extension at 72°C with a 45 s ramp back to 94°C. Alu-PCR amplification products were labeled by incorporation of biotin-11-dUTP (Sigma) by nick translation. DNAs in the range of 50–800 bp with a median size of about 200 bp were used for the in situ hybridization experiments.

2.4. In situ hybridization and probe detection

Prometaphase chromosome spreads were obtained from PHA-stimulated normal male human lymphocytes using standard procedures [16], a BudR cocktail being used instead of thymidine as a release, to enable replication banding to be done. In situ hybridization and washing procedures were performed essentially as described by Lichter and Cremer [17] with modification. Briefly, pretreatment of the chromosome slides was for 30–60 min at 65°C followed by RNase treatment (100 μg RNase A/ml 2 × SSC, 1 h. at 37°C), 3 rinses in 2 × SSC, pepsin treatment (50 μg/ml Pepsin in 0.01 M HCl, pH 2.3, incubated at 37°C for 10 min) washing in PBS, then fixation in 1% formaldehyde and finally ethanol dehydration. Hybridizations were done with 100 ng of biotin-labeled DNA from the different YACs, plus 80 μg human Cot1 DNA (Gibco-BRL), 5 μg of sheared salmon sperm DNA and 5 μg of *E. coli* tRNA in 10 μl of 10% dextran sulfate, 50% formamide, 2 × SSC, and were denatured at 73°C for 5 min, preannealed for 20 min at 37°C then applied to denatured slides (2 min in 70% formamide/2 × SSC at 70°C followed by ethanol dehydration) under a 20 mm^2 cover glass. Hybridization was overnight at 37°C followed by 3 washes of 5 min in 50% formamide/2 × SSC at 45°C and 0.1 × SSC at 60°C. Slides were blocked with 5% BSA/4 × SSC for 30 min and probe detection was with 10 μg/ml of fluorescein isothiocyanate (FITC) conjugated avidin (Vector Laboratories) in 4 × SSC/0.2% Tween-20 for 30 min. Slides were washed 3 times at room temperature for 5 min in 4 × SSC containing 0.2% Tween-20. Slides were then stained with Hoechst 33258 (0.5 μg/ml) and irradiated for 30 min at a 20 cm distance from two 15 W ultaviolet bulbs. Signal was then amplified using the sandwich technique of Pinkel et al. [18]. Finally slides were stained in DAPI and mounted.

2.5. Image acquisition and enhancement

Slides were examined under a Zeiss Axiovert fluorescent microscope. The DAPI replication banding was viewed with a '02' filter set, the FITC signal with a '09' filter set. Images were captured using a cooled CCD (Photometrics, Tucson, AZ) and displayed on a Macintosh computer. Images were processed as described in Featherstone and Huxley [19]. Chromosomes were identified by the DAPI replicational banding pattern.

3. Results

Using primers derived from the *X. laevis* CK1α cDNA sequence and DNA from a human cDNA library, amplification by PCR yielded the expected fragment of 264 bp. Sequencing of this fragment suggested that the amplification product corresponded to a fragment of the human sequence coding for CK1α since it was highly homologous to the bovine and *X. laevis* sequences. This fragment was labeled and used as a probe to screen the same human cDNA library. Four clones were purified through secondary and tertiary screening. Subcloning of one of these gave a bluescript plasmid containing a 1,600 bp insert. The nucleotide sequence of this insert and the derived amino acid sequence are given in Fig. 1. The coding region defines a protein of 337 amino acids with a M_r of 38,874 Da. This nucleotide sequence is highly homologous to the CK1α species found in bovine and in *X. laevis*. Fig. 2 compares the derived amino acid sequence obtained from the human clone to that of the bovine CK1α, rat CK1δ, and HRH25 of *S. cerevisiae*. A remarkable conservation is noted, since it is identical when compared to bovine CK1α, except that the human protein has 12 additional amino acids at the carboxyl end. At the nucleotide level the human and bovine sequences of the α subspecies are 85% identical.

Using the primers derived from the CK1α bovine sequence as described in section 2, a human YAC library was screened and 3 YACs were found to be positive. With these YACs, and using PCR primers and conditions for amplification detailed in section 2, the expected 102 bp PCR fragment was obtained. Upon sequencing, the fragment from all three YACs and human genomic DNA were found to be consistent with the known amino acid sequence of the human CK1. The fact that these YAC clones contained the CK1 was verified by the fact

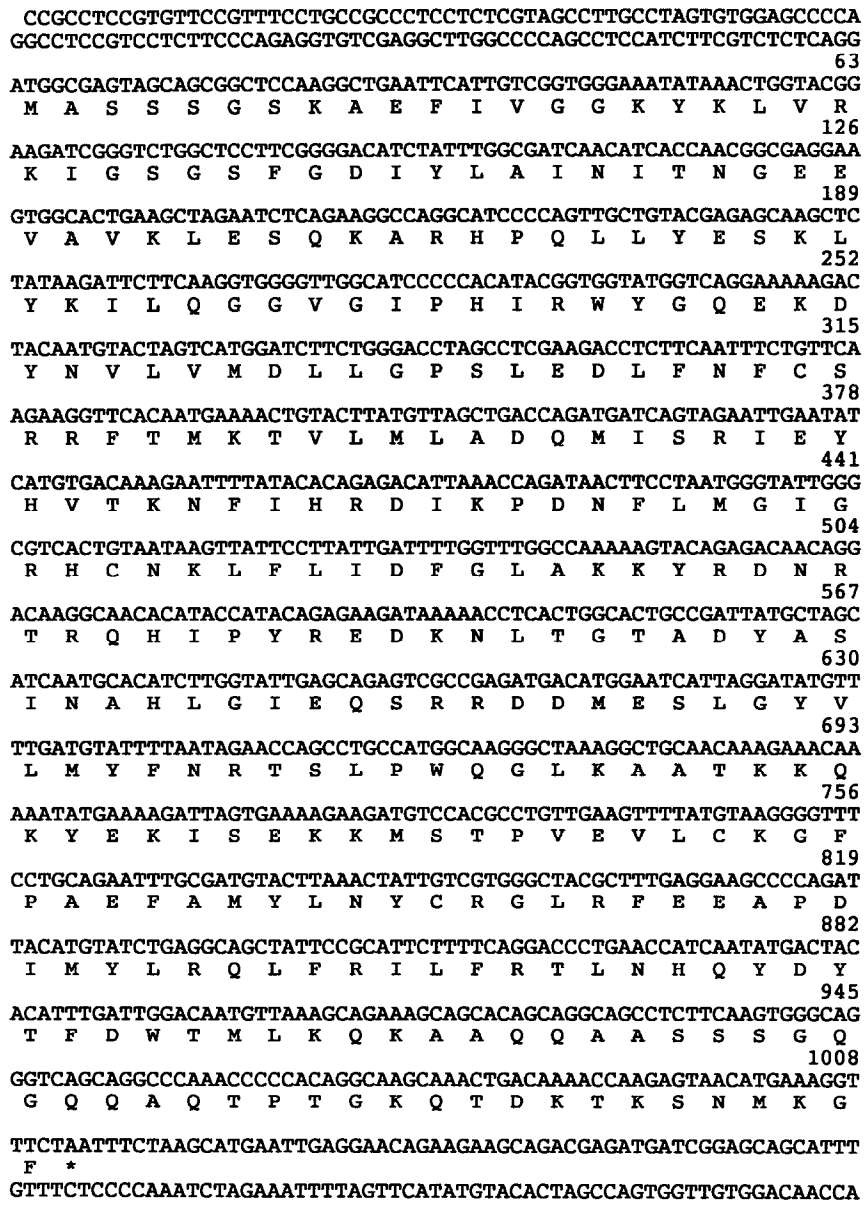

Fig. 1. Deoxynucleotide and derived amino acid sequences of a cDNA coding for human protein kinase CK1α.

that they hybridized with λZAP FA 603, one of the cDNA clones for CK1α (data not shown). The YACs containing the human CK1α gene were used to localize this gene by the use of fluorescent in situ hybridization (FISH). Fig. 3 shows a clear signal in 13q13 where the same location was observed with all the three YACs. Two gave a single signal and did not hybridize with any other region, but the third YAC also gave hybridization on 5q26 and 7p15.2. This YAC clone contained two different size YACs both of which were positive by Southern analysis and hybridization with the cDNA RA 603. This clone would be expected therefore to be chimeric, with the smaller YAC probably being a rearrangement of the larger.

4. Discussion

This communication reports the first cloning of a human gene coding for CK1. The amino acid sequence derived from the cDNA nucleotide sequence demonstrates that this clone corresponds to the α subspecies of CK1 described in bovine brain [6]. Comparison of these sequences demonstrates a remarkable degree of conservation since there are no differences observed throughout the amino acid sequences, except that the human protein has 12 additional amino acids at the carboxyl end. The human CK1α has all the signature sequences conserved among all the CK1s so far described such as LLGPSLEDLF (residues 92–100), HIPXR (residues 172–176),

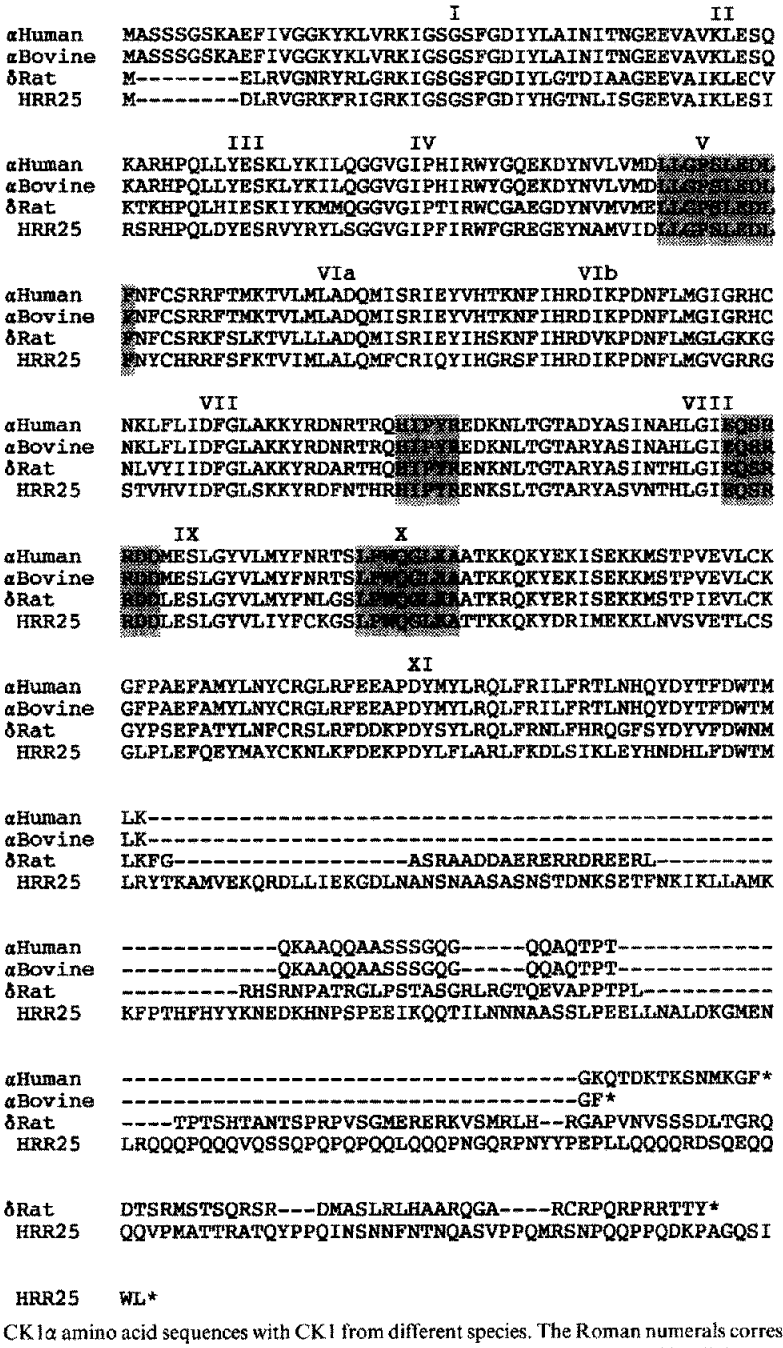

Fig. 2. Comparison of human CK1α amino acid sequences with CK1 from different species. The Roman numerals correspond to the conserved regions present in the catalytic domain of protein kinases [27]. The shaded areas define regions that are conserved in all CK1 sequences [8]. Hyphens indicate spaces to optimize alignment.

EXSRRDD (residues 197–203) and LPWQGLKA (residues 219–226) as pointed out by Graves et al. [7].

The high level of conservation points to a protein with important, probably essential, cellular functions that tolerate little structural variations. It is interesting that the human protein has an extended carboxyl tail in view of the fact that it has been recently demonstrated that extension in this terminal segment *S. pombe* in CK1 serves to down-regulate the activity of the enzyme [20].

This work did not attempt to look exhaustively for other members of the CK1 family in the human genome. However, two separately isolated cDNA clones, detected with a probe to a region that is common to all the bovine isoforms, gave identical nucleotide sequences. The partial nucleotide sequence obtained from PCR fragment from the YACs also corresponded to the same α species. This may suggest that other members of the CK1 family in the human may be sufficiently divergent not to be

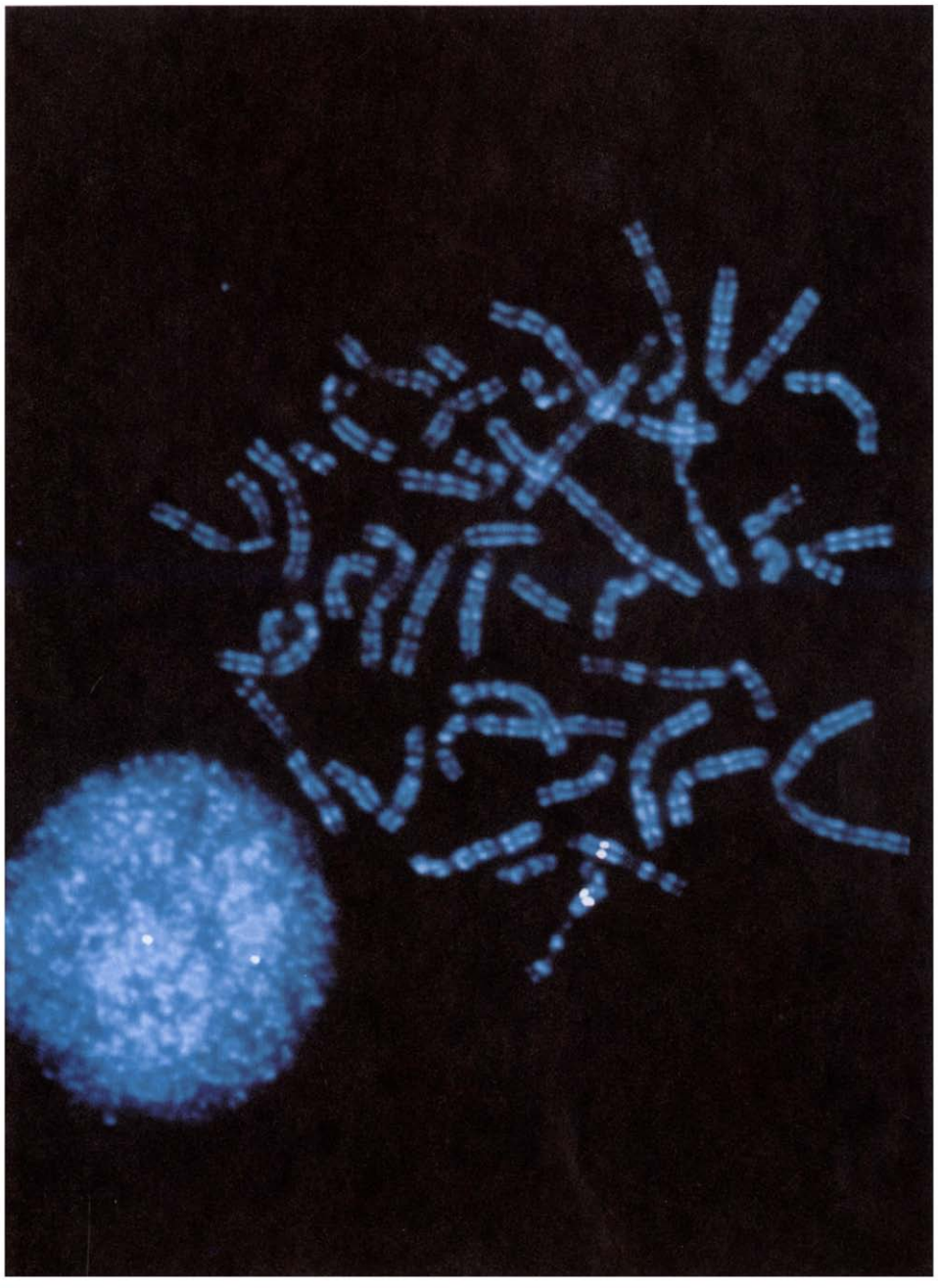

Fig. 3. A replication banded human metaphase showing hybridization of a CK1 YAC to chromosome 13q13.

detected with the probes used or that these members are poorly expressed or not present in the libraries that were screened.

The gene coding for the α species of human CK1 is located in band q13 of chromosome 13. It is of considerable interest that in the vicinity of the CK1α locus is the retinoblastoma locus which maps to 13q14.1–13q14.2 [21]. In this same area is the gene for esterase D which maps to 13q14.11 [22]. FMS-like tyrosine kinases-1 and -3 both map to 13q12 [23] and Moebius syndrome (congenital facial diplegia) map to 13q12.2-q13 [24]. Some observations suggest a non-random involvement of band 13q13 in chronic lymphoproliferative disease [25]. Of special interest is a previous report of an X-ray sensitivity locus in 13q14 [26] as there may be a relation to experiments in yeast that involve CK1 in DNA repair [5].

The cloning and localization of the human CK1α gene should be helpful in defining the in vivo function(s) of this enzyme.

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